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Claim 17, line 1, change "any one of the preceding claims" to --claim 1--.

Claim 19, line 1, change "any one of the preceding claims" to --claim 1--.

Claim 21, lines 1-2, change "any one of the preceding claims" to --claim 1--.

Please cancel Claim 27 without prejudice or disclaimer.

Claim 28, lines 1-2, change "any one of claims 1 to 20" to --claim 1--.

Claim 29, lines 1-2, change "any one of claims 1 to 20" to --claim 1--.

REMARKS

The above amendments are made to reduce initial filing fees by removing multiple dependent claims.

Respectfully submitted,

NIXON & VANDERHYE P.C.

By: _____

Arthur R. Crawford
Reg. No. 25,327

ARC:ms
1100 North Glebe Road, 8th Floor
Arlington, VA 22201-4714
Telephone: (703) 816-4000
Facsimile: (703) 816-4100

Claims

1. A protein having luciferase activity and at least 60% similarity to luciferase from *Photinus pyralis*, *Luciola mingrelica*, *Luciola cruciata*, *Luciola lateralis*, *Hotaria paroula*, *Pyrophorus plagiophthalamus*, *Lampyris noctiluca*, *Pyrocoelia nayako* or *Photinus pennsylanvanica*, wherein in the sequence of the enzyme, at least one of

(a) the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase or to residue 216 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase;

(b) the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase or to residue 234 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase;

(c) amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase or to residue 297 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase;

(d) amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase or to residue 16 of *Luciola mingrelica*, or 17 of *Luciola cruciata* or *Luciola lateralis*;

(e) amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase or to residue 37 of *Luciola mingrelica*, or 38 of *Luciola cruciata* or *Luciola lateralis*;

(f) the amino acid residue corresponding to amino acid residue 105 of the *Photinus pyralis* luciferase or to residue 106 of *Luciola mingrelica*, 107 of *Luciola cruciata* or *Luciola lateralis* or 108 of *Luciola lateralis* gene;

(g) amino acid residue corresponding to amino acid residue 234 of the *Photinus pyralis* luciferase or to residue 236 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis*;

(h) amino acid residue corresponding to amino acid residue 420 of the *Photinus pyralis* luciferase or to residue 422 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis*;

(i) amino acid residue corresponding to amino acid residue 310 of the *Photinus pyralis* luciferase or to residue 312 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis*;

is different to the amino acid which appears in the corresponding wild type sequence and wherein the luciferase enzyme has increased thermostability as compared to an enzyme having the amino acid of the corresponding wild-type luciferase at this position.

2. A protein according to claim 1 which has the sequence of a wild-type luciferase, in which more than one amino acid residue is different to that of the wild type enzyme.

3. A protein according to claim 2 wherein up to 50 amino acids are different to that of the wild type enzyme.

4. A protein according to ~~any one of the preceding claims~~ claim 1 wherein the luciferase is a modified form of luciferase of *Photinus pyralis*, *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase.

5. A protein according to ~~any one of the preceding claims~~ claim 1 wherein the sequence of luciferase of *Photinus pyralis*, wherein at least one of

(a) the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase is other than threonine;

(b) the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase is other than isoleucine;

(c) amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase is other than phenylalanine;

(d) amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase is other than phenylalanine;

(e) amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase is other than leucine;

(f) amino acid residue corresponding to amino acid residue 105 of the *Photinus pyralis* luciferase is other than alanine;

(g) amino acid residue corresponding to amino acid residue 234 of the *Photinus pyralis* luciferase is other than aspartic acid;

(h) amino acid residue corresponding to amino acid residue 420 of the *Photinus pyralis* luciferase is other than serine;

(i) amino acid residue corresponding to amino acid residue 310 of the *Photinus pyralis* luciferase is other than histidine.

6. A protein according to ~~any one of claims 1 to 4~~ Claim 1 wherein protein has substantially the sequence of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* enzyme, and wherein at least one of

(a) the amino acid residue corresponding to residue 216 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is other than glycine (for *Luciola mingrelica* based sequences) or aparagine (for *Luciola cruciata* or *Luciola lateralis*) based sequences;

(b) the amino acid residue corresponding to residue 234 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is other than serine;

(c) amino acid residue corresponding to residue 297 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is other than leucine;

(d) amino acid residue corresponding to amino acid 16 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* is other than phenylalanine;

(e) amino acid residue corresponding to residue 37 of *Luciola mingrelica*, or residue 38 of *Luciola cruciata* and *Luciola lateralis* is other than lysine;

(f) amino acid residue corresponding to amino acid residue 106 of *Luciola mingrelica*, 107 of *Luciola cruciata* or *Luciola lateralis*, or 108 of *Luciola lateralis gene* is other than glycine;

(g) amino acid residue corresponding to amino acid residue 236 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* is other than glycine;

(h) amino acid residue corresponding to residue 422 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* is other than threonine;

(i) amino acid residue corresponding to amino acid residue 312 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* is other than threonine (for *Luciola mingrelica* based sequences)

or valine (for *Luciola cruciata* or *Luciola lateralis*) based sequences.

7. A protein according to ~~any one of the preceding claims~~ Claim 1 wherein comprising a protein having luciferase activity and at least 60% similarity to luciferase from *Photinus pyralis*, *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* enzyme wherein in the sequence of the enzyme, at least one of

(a) the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase and to residue 216 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is mutated and is other than threonine in the case of *Photinus pyralis* luciferase; or

(b) the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase and to residue 234 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is mutated and is other than isoleucine in the case of *Photinus pyralis* luciferase; or

(c) amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase and to residue 297 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is mutated and is for example, other than phenylalanine in the case of *Photinus pyralis* luciferase;

and the luciferase enzyme has increased thermostability as compared to the wild-type luciferase.

8. A protein according to claim 1 wherein the amino acid residue corresponding to residue 214 in *Photinus pyralis* luciferase and to residue 216 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is alanine.

9. A protein according to ~~any one of the preceding claims~~ Claim 1 wherein the amino acid residue corresponding to residue 232 in *Photinus pyralis* luciferase and to residue 234 of *Luciola mingrelica*, *Luciola cruciata* or *Luciola lateralis* luciferase is alanine.

10. A protein according to ~~any one of the preceding claims~~ Claim 1 which is a mutated *Photinus pyralis* luciferase wherein the amino acid residue corresponding to residue 295 in *Photinus pyralis* luciferase is leucine.

11. A protein according to ~~any one of the preceding claims~~ Claim 1 wherein the amino acid residue corresponding to amino acid 14 of the *Photinus pyralis* luciferase or to amino acid 16 in Luciola luciferase, is alanine.

12. A protein according to ~~any one of the preceding claims~~ Claim 1 wherein the luciferase is a mutated luciferase of *Photinus pyralis* or a *Luciola* species where the amino acid residue corresponding to amino acid 35 of the *Photinus pyralis* luciferase or to amino acid residue 37 in *Luciola mingrelica* or 38 of *Luciola lateralis* or *cruciata* luciferase is alanine.

13. A protein according to ~~any one of the preceding claims~~ Claim 1 wherein the amino acid residue corresponding to residue 105 in *Photinus pyralis* luciferase and to residue 106 of *Luciola mingrelica*, 107 of *Luciola cruciata* or *Luciola lateralis* or 108 of *Luciola lateralis gene* luciferase is valine.

14. A protein according to ~~any one of the preceding claims~~ Claim 1 which comprises a mutated *Photinus pyralis* luciferase wherein the amino acid residue corresponding to residue 234 in *Photinus pyralis* luciferase is glycine.

15. A protein according to ~~any one of the preceding claims~~ Claim 1 which comprises a mutated *Photinus pyralis* luciferase wherein the amino acid residue corresponding to residue 420 in *Photinus pyralis* luciferase is threonine.

16. A protein according to ~~any one of the preceding claims~~ Claim 1 which comprises a mutated *Photinus pyralis* luciferase wherein the amino acid residue corresponding to residue 310 in *Photinus pyralis* luciferase is arginine.

17. A protein according to ~~any one of the preceding claims~~ Claim 1 wherein the amino acid at position corresponding to amino acid 354 of the *Photinus pyralis* luciferase (356 in Luciola luciferase) is other than glutamate.

18. A protein according to claim 17 wherein the amino acid at position corresponding to amino acid 354 of the *Photinus pyralis* luciferase (356 in Luciola luciferase) is lysine or arginine.

19. A protein according to ~~any one of the preceding claims~~ Claim 1 wherein the amino acid at the position corresponding to amino acid 217 in Luciola luciferase (215 in *Photinus pyralis*) is a different hydrophobic amino acid.

20. A protein according to claim 19 wherein the amino acid at the position corresponding to amino acid 217 in Luciola luciferase (215 in *Photinus pyralis*) is isoleucine, leucine or valine.

21. A nucleic acid which encodes a luciferase according to ~~any one of the preceding claims~~ Claim 1.

22. A vector comprising a nucleic acid according to claim 21.

23. A cell transformed with a vector according to claim 22.

24. A cell according to claim 23 which is a prokaryotic cell.

25. A cell according to claim 23 which is a plant cell.

26. A plant comprising cells according to claim 25.

27. A method of producing a protein according to any one of claims 1 to 20, which method comprises culture of a cell according to claim 23 or growth of a plant according to claim 26.

28. The use of a protein according to ~~any one of claims 1 to 20~~ Claim 1 in a bioluminescent assay.

29. A kit comprising a protein according to ~~any one of claims 1 to 20~~ Claim 1.

30. A kit according to claim 29 which further comprises luciferin.

Fig.5.

CGCCGGTGAGCTCCCCGCCGCCG SACI-SENSE / 6371
CGGCGGCGGGGAGCTCACCGGCG SACI-ANTI / 6372
CGAACACTTCTTCATCGTTGACCGCCTTAAGTCTTTAATTAAATACAAAGG AFLII-SENSE / 6373
CCTTTGTATTTAATTAAAGACTTAAGGCGGTCAACTATGAAGAAGTGTTCG AFLII-ANTI / 6374
GAAAGGCCCGGCACCAGCCTATCCTCTAGAGG F14A-SENSE / 6375
CCTCTAGCGGATAGGCTGGTGCCGGGCCTTTC F14A-ANTI / 6376
CCATAAATTTACCGAATTCGTCGACTTCGATCGAGG C-TERM.SEQ/ 6641
GTGTGGAATTGTGAGCGG N-TERM.SEQ / 6651
GAGATACGCCGCGGTTCCTGG L35A-SENSE / 6652
CCAGGAACCGCGGCGTATCTC L35A-SENSE / 6653

CCCTATTTTCATTCCTGGCCAAAAGCACTG F295L-SENSE/ 9048
GAGTGCTTTTGGCCAGGAATGAAAATAGGG F295L-ANTI / 9049
CCGCATAGAgCTCTCTGCGTCAGATTC T214A + A215L-SENSE / 9063
GAATCTGACGCAGAGAGcTCTATGCGG T214A + A215L-ANTI / 9064
GTTGACCGCTTGGGATCCTTAATTAAATAC Insertion of BamHI at G339 / 9077

GTATAGATTTGAAAAAGAGCTG E270K-SENSE / 257
CAGCTCTTTTTCAAATCTATAC E270K-ANTI / 258
GGCTACATACTGGAGACATAGC S420T-SENSE / 629
GCTATGTCTCCAGTATGTAGCC S420T-ANTI / 630
GCAGTTGCGCCCGTGAACGAC A105L-SENSE / 790
GTCGTTCACGGGCGCAACTGC A105L-ANTI /791
CAAATCATTCCGGGTACTGCGATTTTAAG D234G-SENSE / 792
CTTAAAATCGCAGTACCCGGAATGATTTG D234G-ANTI / 793

CCGCATAGAACTCTCTGCGTCAGATTC A215L-SENSE / 7726
GAATCTGACGCAGAGAGTTCTATGCGC A215L-ANTI / 7727
CTGATTACACCCAAGGGGGATG E354K-SENSE / 7792
CATCCCCCTTGGGTGTAATCAG E354K-ANTI / 7793
ccctteegcatagannngcctgcgtcagt T214N-Sense / 8202
actgacgcaggcNNNtctatgcggaaggg T214N-Anti / 82033

GCAATCAAATCGCTCCGGATACTGC I232A-SENSE / 6911
GCAGTATCCGGAGCGATTTGATTGC I232A-ANTI / 6912

CCATTCCATCAAGGTTTTGG H245Q-SENSE / 9128

CCAAAACCTTGATGGAATGG H245Q-ANTI / 9129